

## Confirming *Agrobacterium* with Benedict's Reagent

- Prepare a streak of the culture being confirmed on Lactose Agar. It is not necessary to include selective agents in the medium but allow 2-3 days growth since young cultures tend to have weak responses.
- Flood all or part of the plate with Benedict's Reagent (1 to 5 ml per plate is adequate).
- Wait for up 1-2 hours or until a bright, yellow product is observed in the agar. The yellow color confirms the presence of *Agrobacterium* sp. since *E. coli* does not convert lactose to 3-ketolactose. Absence of the yellow color may mean that the culture in question is, in fact, something other than an *Agrobacterium*. (A few biovars of *Agrobacterium* are unable to carry out the conversion).

### Reagents:

#### **Benedict's Solution<sup>1</sup>**

##### **- Solution I**

Mix the following:            17.3g Na Citrate  
   10.0g Na<sub>2</sub>CO<sub>3</sub>  
   60-70ml **hot** deionized water (~55 deg.)

##### **Solution II**

1.73g /10 ml H<sub>2</sub>O CuSO<sub>4</sub> · 5H<sub>2</sub>O

Cool **Solution I**, add 10 ml **Solution II**, bring up to 100ml with deionized water. Stable at room temperature.

#### **Lactose Agar**

*(This is NOT the same thing as L-Agar or LB-Agar)*

Mix the following:            1g yeast extract  
   10g lactose  
   15g Agar

- add 1 liter deionized water.
- autoclave for 20 min.
- let cool to 55°C and pour into Petri dishes

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<sup>1</sup> Bernaerts M.J. and De Ley J. (1963) A biochemical test for crown gall bacteria. Nature 167:406-407.